A Novel Dominant Transformer Allele of the Sex-Determining Gene *her-1* of *Caenorhabditis elegans*

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ABSTRACT

We have characterized a novel dominant allele of the sex-determining gene *her-1* of *Caenorhabditis elegans*. This allele, called *n695*, results in the incomplete transformation of *XX* animals into phenotypic males. Previously characterized recessive *her-1* alleles transform *XO* animals into phenotypic hermaphrodites. We have identified five new recessive *her-1* mutations as intragenic suppressors of *n695*. Three of these suppressors are weak, temperature-sensitive alleles. We show that the recessive *her-1* mutations are loss-of-function alleles, and that the *her-1(n695)* mutation results in a gain-of-function at the *her-1* locus. The existence of dominant and recessive alleles that cause opposite phenotypic transformations demonstrates that the *her-1* gene acts to control sexual identity in *C. elegans*.

In many species, the chromosomal complement of an organism plays a primary role in the determination of sex: the presence, absence or relative numbers of particular chromosomes specify the sexual phenotype. Genetic studies in a variety of species [*e.g.*, the fruit fly (BAKER and BELOTE 1983); the mouse (EICHER and WASHBURN 1986); and the nematode (see below)] have shown that individual genes can have crucial roles in this process, that is, mutations in specific genes can have profound effects on the development of sexual phenotype. The analysis of such genes and how they control developmental decisions should provide insight into the mechanisms involved in sex determination and the genetic control of alternative pathways of differentiation.

The free-living nematode *Caenorhabditis elegans* has a hermaphrodite sex (with two sets of five autosomes and two *X* chromosomes: *2A;XX*) and a male sex (with two sets of five autosomes and one *X* chromosome: *2A;XO*), which show substantial sexual dimorphism (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON *et al.* 1983). Mutations in a number of autosomal genes have been identified that affect sex determination in *C. elegans*: transformer mutations transform *XX* animals into phenotypic males (Tra phenotype); hermaphroditization mutations transform *XO* animals into phenotypic hermaphrodites (Her phenotype); and feminization mutations transform *XX* and *XO* animals into females, that is, spermless hermaphrodites (Fem phenotype). Specifically, recessive transformer mutations define the genes *tra-1 III*, *tra-2 II* and *tra-3 IV* (HODGKIN and BRENNER 1977); recessive hermaphroditization mutations define the gene *her-1 V* (HODGKIN 1980); and recessive feminization mutations define the genes *fem-1 IV*, *fem-2 III*, and *fem-3 IV* (HODGKIN 1986; DONIACH and HODGKIN 1984; KIMBLE, EDGAR and HIRSH 1984).

The dominant mutation *n695* was isolated and initially characterized as part of a general study of egg-laying defective mutants (TRENT, TSUNG and HORVITZ 1983). The pleiotropic effects of *n695*, that is, apparent partial sexual transformation of *XX* animals, suggested that this mutation has a general effect on the development of sexual phenotype. The genetic experiments described in this paper demonstrate that *n695* acts like a transformer mutation and that it is allelic with the recessive *her* mutations that define the gene *her-1*.

MATERIALS AND METHODS

Strains and genetic nomenclature: *Caenorhabditis elegans* var. Bristol strain N2 was the wild-type parent of all strains used in this work. The following genes and alleles were used:

Linkage Group (LG) I: *dpy-5(e61)*, *unc-13(e450, e1091)*;
LG II: *dpy-10(e128)*;
LG III: *lon-1(e185)*, *unc-36(e251)*, *sup-5(e1464)*, *unc-32(e189)*, *dpy-18(e364)*;
LG IV: *unc-5(e53)*, *him-8(e1489)*;
LG V: *dpy-11(e224)*, *unc-23(e25)*, *her-1(e1518, e1519, e1520, e1558, e1559, e1564, e1574, e1807, e1821, e1914, e1917, y10, y14)*, *unc-42(e270)*, *unc-41(e268)*, *egl-3(n150ts)*, *daf-11(m47ts)*, *sma-8(n716dm)*, *sma-1(e30)*, *him-5(e1490, e1467)*, *dpy-21(e428, e459)*;
LG X: *dpy-3(e27)*, *lon-2(e678)*, *sup-7(st5)*.

egl-3 has been described by TRENT, TSUNG and HORVITZ (1983); *dpy-21* by HODGKIN and BRENNER (1977); *her-1* by HODGKIN (1980); *him-5* and *him-8* by HODGKIN, HORVITZ and BRENNER (1979); *sup-5* by WATERSTON and BRENNER (1978) and WILLS *et al.* (1983); *sup-7* by WATERSTON (1981); *daf-11* by RIDDLE, SWANSON and ALBERT (1981); and the

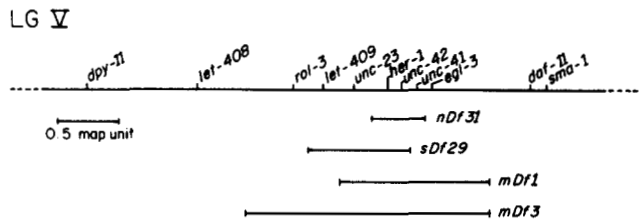

FIGURE 1.—Partial genetic map of linkage group V of *C. elegans*. The map positions and map distances are based upon the *Caenorhabditis* Genetics Center's 1987 *C. elegans* Genetic Map (EDGLEY and RIDDLE 1987) and data in this paper. The map position of *egl-3* and the end points of *nDf31* are based on mapping and complementation experiments presented in MATERIALS AND METHODS. The end points of *sDf29* have been defined by ROSENBLUTH, CUDDEFORD and BAILLIE (1985) and by R. ROSENBLUTH (personal communication); we have further defined the right end point by finding that *sDf29* complements *unc-41*. The end points of *mDf1* and *mDf3* were defined by S. BROWN, D. RIDDLE and R. ROSENBLUTH (personal communications); in addition, we have shown that both *mDf1* and *mDf3* fail to complement *egl-3*.

other mutants by BRENNER (1974). The *her-1* alleles *y10* and *y14* were isolated by J. PLENEFISCH and B. MEYER (personal communication). The translocation *eT1(III;V)*, which fails to complement *unc-36*, has been described by ROSENBLUTH and BAILLIE (1981). The deficiencies *mDf1 V* and *mDf3 V* were isolated by S. BROWN (personal communication); the deficiency *sDf29 V* was described by ROSENBLUTH, CUDDEFORD and BAILLIE (1985). Figure 1 shows a partial genetic map of LG V.

Genetic nomenclature of this paper conforms to the standard conventions for *C. elegans* (HORVITZ *et al.* 1979). Intragenic revertants of *n695* are named as double mutants: for example, *n826n695* [see GREENWALD and HORVITZ (1980) for an explanation of this nomenclature]. The class of recessive *her-1* mutations is indicated generally as *her-1(r)*.

General methods: General techniques for the culture, genetic analysis, and ethylmethanesulfonate (EMS) mutagenesis of *C. elegans* have been described by BRENNER (1974) and HERMAN and HORVITZ (1980). Gamma-ray mutagenesis of *C. elegans* was performed as described by GREENWALD and HORVITZ (1980) or MENEELY and WOOD (1984). Experiments were performed at 20° unless otherwise indicated.

***her-1(n695)* Genetics:** *n695* was positioned between *unc-23* and *unc-42* by standard 3-factor crosses: (1) six out of nine Dpy non-Egl non-Tra recombinants picked from the progeny of *dpy-11 n695/unc-23* heterozygotes segregated Unc hermaphrodites, and (2) three out of 73 Unc non-Dpy recombinants picked from the progeny of *dpy-11 unc-42/n695* heterozygotes segregated Egl-Tra animals. [For convenience, in the MATERIALS AND METHODS section the variable *XX* phenotype resulting from the *n695* mutation (see description in RESULTS) is designated as Egl-Tra. *XX* animals not exhibiting this phenotype are designated as non-Egl non-Tra.]

n695/+ heterozygotes were generated by crossing *n695* males with *dpy-11* or *unc-42* hermaphrodites or by crossing N2 males with *dpy-11 n695* or *dpy-10; unc-36; n695* hermaphrodites. *n695/her-1(r)* heterozygotes were generated by crossing *n695* males with *dpy-11 her-1(e1520)*, *unc-42 her-1(e1520)* or *dpy-11 her-1(e1518)* hermaphrodites. *n695/Df* heterozygotes were generated by crossing *Df/eT1* males with *dpy-10; unc-36; n695* hermaphrodites: all non-Unc cross progeny animals were *n695/Df*. An extensive series of experiments was performed examining the phenotypes of *n695/+* and *n695/her-1(r) XX* animals. Over 3000 *n695/+* heterozygotes were scored in several experiments; 50% of these showed a mutant phenotype. Over 2000 *n695/her(r)* animals were scored in several experiments; again, approximately 50% were phenotypically mutant. It should be noted that the percentage of such *n695/+* and *n695/her-1(r)* heterozygotes that appear mutant often varies from experiment to experiment.

The frequency of nullo-*X* ovum production by *her-1(n695)* hermaphrodites was determined using a protocol similar to that described by HODGKIN, HORVITZ and BRENNER (1979). *lon-2 X* males were crossed with *n695 V; dpy-3 X* hermaphrodites and the cross progeny were scored for patroclinous Lon-2 males, which would result from fertilization of nullo-*X* ova from *n695; dpy-3* hermaphrodites with sperm from *lon-2* males. No Lon-2 males were observed among approximately 1000 cross progeny (non-Lon, non-Dpy) hermaphrodites. [Among the cross progeny were four non-Lon, non-Dpy males, three of which were slightly abnormal. These males must have been *XX* in genotype since they were neither Dpy nor Lon.] These experiments indicate that *n695* hermaphrodites product nullo-*X* ova at a frequency [estimated as patroclinous males (*XO*)/patroclinous males + cross progeny hermaphrodites] of less than 0.1%. As a control, nullo-*X* ovum production by *him-5(e1467)* was measured by crossing *lon-2 X* males with *him-5 V; dpy-3 X* hermaphrodites and the progeny scored for patroclinous Lon males. Among approximately 980 cross progeny hermaphrodites, 134 Lon-2 males were observed. These results indicate a nullo-*X* ovum production by *him-5(e1467)* hermaphrodites of 12%, in agreement with the determination made by HODGKIN, HORVITZ and BRENNER (1979).

Identification and preliminary genetic analysis of *n695* suppressors: Suppressors of *n695* were isolated by two different protocols. First, 14 revertant strains were generated by picking phenotypically wild-type (non-Egl non-Tra) hermaphrodites from the F_2 progeny of EMS-mutagenized *n695* or *him-8 IV; n695 unc-42 V* hermaphrodites. Second, eight revertant strains were generated by picking phenotypically wild-type hermaphrodites from the F_1 progeny of γ-ray-mutagenized *n695* hermaphrodites. All these revertant strains were initially analyzed with respect to (1) linkage of the suppressor (*sup*) mutation to *n695*, and (2) complementation of the suppressor mutation with a *her-1(r)* mutation. Linkage was tested by scoring the progeny of *revertant/+* heterozygotes (*sup/+; n695/+* or *sup n695/+ +*) for segregation of nonrevertant (*i.e.*, Egl-Tra) animals. Complementation with the recessive *her-1* alleles *e1518* or *e1520* was tested using the following protocol, which is similar to that described by HODGKIN (1980). *Revertant/+* males were crossed with *unc-5 IV; her-1(r) V; lon-2 X* hermaphrodites and the cross progeny (*i.e.*, non-Unc animals) were scored for Lon-2 hermaphrodites. If the suppressor in the revertant strain failed to complement *her-1*, then ¼ of the cross progeny should be hemizygous (*XO*) Lon-2 hermaphrodites.

Based on the above analysis, revertant strains were designated as carrying either intragenic or extragenic suppressor mutations. With the first protocol, the intragenic suppressors *n826, n827, n830* and *n1100* were obtained; with the second, *ct22* was obtained. Each of these mutations was very closely linked to *n695* and each failed to complement *her-1(e1520)* or *her-1(e1518)*. All the extragenic suppressor mutations (listed below) complemented *her-1(r)* mutations and were easily separated by recombination from the *n695* mutation.

Using the first protocol, the following extragenic suppressor mutations were obtained: *sup-26(n1091) III, sup-*

27(n1092) V, *sup-27(n1102) V*, *n828*, *n829*, *n831*, *n832*, *n1090*, *n1103* and *n1104*. Using the second protocol, the extragenic suppressor mutations *ct24*, *ct25*, *ct27*, *ct28 X*, *ct29*, *ct30 X* and *ct31 X* were identified. For most of the suppressor mutations listed, the *sup*; *n695* double mutant strain is essentially wild type with respect to hermaphrodite sexual phenotype although some strains exhibit an egg-laying defective phenotype. Revertant strains containing the suppressor mutations *ct27*, *ct28*, *ct29*, *ct30* and *ct31* have a dumpy body shape (Dpy). *n695* hermaphrodites of genotype *2A;3X* are Dpy and do not exhibit the partial sexual transformation characteristic of *n695* animals (TRENT 1982); thus the Egl-Tra phenotype resulting from *n695* is suppressed by the presence of an extra *X* chromosome. Such *2A;3X n695* strains segregate Dpy (*XXX*) and non-Dpy (*XX*) animals; the latter are phenotypically Egl-Tra. The Dpy revertant strains listed above do not behave genetically as triplo-*X* strains, although a number of apparent triplo-*X* revertant strains were generated using the second protocol.

Linkage of intragenic suppressor mutations to the *n695* mutation: For all five intragenic revertant strains, linkage of the suppressor mutation to *n695* was more precisely determined by constructing *her-1(r) n695/+ +* heterozygotes and scoring large numbers of progeny for animals with the Egl-Tra phenotype, that is, *n695* recombinants lacking the *her-1(r)* mutation.

n826 and *n827*: No Egl-Tra animals were observed among approximately 3000 progeny segregating from *n826n695/+ +*; *lon-2/+* heterozygotes or among 2900 progeny segregating from *n827n695/dpy-11 unc-42* heterozygotes. Taking into account that *n695* is semi-dominant (that is, about 50% of either *n695 +/her-1(r)n695* or *n695 +/+ + XX* animals will be scored as phenotypically Egl-Tra), the number of *n695* recombinants/total progeny would be equal to $p/2$ for small p, where p is the recombination frequency. Thus the map distance between *n826* and *n695* or *n827* and *n695* is <0.07%.

ct22: Among 5400 progeny segregating from *dpy-11 ct22n695/+ + +* hermaphrodites, no Egl-Tra animals were observed. Thus, the map distance between *ct22* and *n695* is <0.04%.

n830: Among 3600 progeny segregating from *+ n830n695 +/dpy-11 + + unc-42* hermaphrodites, no Egl-Tra animals were observed. Thus, the map distance between *n830* and *n695* is <0.06%.

n1100: Among 3600 progeny segregating from *him-8/+*; *n1100n695 unc-42/+ + +* hermaphrodites, no Egl-Tra non-Unc animals were observed. Since many *n1100n695 unc-42* hermaphrodites are egg-laying defective (Egl), only non-Unc animals were scored for the Egl-Tra recombinant phenotype. If the order of mutations is *n1100 n695 unc-42*, then *n695* non-*n1100* recombinants would be of genotype *+ n695 unc-42/n1100n695 unc-42* or *+ n695 unc-42/+ + +*. Taking into account that 50% of the latter recombinants would exhibit an Egl-Tra phenotype, the number of non-Unc *n695* recombinants/total progeny would be equal to $p/4$. The estimated recombination distance between *n1100* and *n695* would then be <0.1%. If the order of mutations is *n695 n1100 unc-42*, the estimated distance would be <0.05%.

Examination of *her-1 XO* and *her-1/Df XO* animals carrying strong *her-1(r)* alleles (*e1520*, *e1518*, *n827*, *ct22*): To examine the phenotypes of *n827n695* and *ct22n695 XO* animals, double mutant strains carrying a *him-8 IV* mutation were constructed. For the *n827n695* construction, *him-8*; *unc-42/+* males were crossed with *n827n695* hermaphrodites; from the progeny of *him-8/+*; *n827n695/unc-42* heterozygotes, non-Unc hermaphrodites were picked. Those animals that segregated males and Unc's were of genotype *him-8*; *n827n695/unc-42*; from their progeny non-Unc hermaphrodites were picked and their progeny scored for Unc animals. Hermaphrodites that did not segregate Unc animals were of genotype *him-8*; *n827n695*. For the *ct22n695* construction, *him-8*; *lon-2* males were crossed with *dpy-11 ct22n695* hermaphrodites. From the progeny of *him-8/+*; *dpy-11 ct22n695/+ + +*; *lon-2/+* heterozygotes, Lon animals were picked; from the progeny of *him-8*; *dpy-11 ct22n695/ + + +*; *lon-2* hermaphrodites, Dpy animals were saved. These animals should be of genotype *him-8*; *dpy-11 ct22n695*; *lon-2*. (The *lon-2* mutation was included in this strain for use in the construction of a *ct22n695*; *sup-7* strain.)

her-1(r)/Df XO heterozygotes were made by crossing *Df/eT1* males with a triply mutant strain *m*; *her-1(r)*; *lon-2 X*, where *m* is a marker used to distinguish cross-progeny from self progeny. Lon non-Marker hermaphrodites are of genotype *m/+*; *her-1/Df*; *lon-2/O*. *m/+*; *her-1(r)*; *lon-2/O* animals were also generated by using *her-1(r)/+* males in the above cross.

Examination of *her-1 XO* and *her-1/Df XO* animals carrying weak *her-1(r)* alleles (*n826*, *n830*, *n1100*): The phenotypes of *her-1 XO* animals of *n826*, *n830* and *n1100* genotypes were initially examined using double mutant strains containing *him-8* and the *her-1* mutation. For the *him-8*; *n826n695* construction, a procedure identical to that for *n827n695* was used. A *him-8*; *n830n695* strain was constructed with a similar protocol using *eT1* as the balancer.

The phenotypes of *n826n695* and *n830n695 XO* animals were also examined by crossing *her-1* males or *him-8*; *her-1* males (for both *n826n695* and *n830n695*, most *XO* animals are wild-type males at 16° and 20°) with *m*; *her-1*; *lon-2 X* hermaphrodites (carrying the same *her-1* mutation as the males). *m* was either *dpy-10* or *unc-36*. Lon non-M animals resulting from such a cross would be homozygous *her-1 XO* animals. Similar crosses were used to determine the phenotype of *XO* animals heterozygous for *n826n695* or *n830n695* and other *her-1(r)* alleles. For example, to generate *n826n695/ct22n695 XO* animals, *n826n695* males were crossed with *dpy-11 ct22n695*; *lon-2* hermaphrodites. (*n826n695/e1520 XO* heterozygotes were also generated using a different cross described below.) To examine *n1100 XO* animals, *him-8/+*; *n1100n695 unc-42/+ + +* males were crossed with *dpy-10*; *n1100n695 unc-42*; *lon-2* hermaphrodites and Lon Unc non-Dpy animals were scored.

For both *n826* and *n830*, *her-1/Df XO* heterozygotes were generated by crossing *Df/eT1* males with *unc-36*; *her-1*; *lon-2* hermaphrodites. All Lon non-Unc's are *XO* animals of genotype *her-1/Df*. (A similar cross with *her-1(e1520)/eT1* males was used to generate *XO* heterozygotes of genotype *e1520/n826n695* and *e1520/n830n695*.) To examine *n1100n695/Df XO* animals, *Df/eT1* males were crossed with *dpy-10*; *n1100n695 unc-42*; *lon-2* hermaphrodites; since all the deficiencies used failed to complement *unc-42*, all Lon Unc non-Dpy's were *XO* animals of genotype *n1100n695/Df*.

Tests for amber suppression: The general strategy used to test a *her-1* allele for amber suppression was to construct a *sup*; *her-1 him* strain (where *sup* is *sup-5* or *sup-7*) and to score this strain for the appearance of intersex animals or males. Such strains were constructed and scored at 20° or 23°. The *sup-5* strains were also scored at 16° because the degree of suppression is greater at this temperature. For all constructions, either *him-8(e1489)* or *him-5(e1490)* was used; neither of these mutations is amber suppressible.

The following protocol was used to generate a double mutant containing *sup-5 III* and *her-1(n827n695) V*. Males of genotype *lon-1 sup-5/+ + III*; *him-8 IV*; *unc-42/+ V* were

crossed with *him-8 IV*; *her-1(n827n695) V* hermaphrodites. From the progeny of *lon-1 sup-5/+ +*; *him-8*; *unc-42/her-1* heterozygotes, Lon non-Unc animals were picked. Hermaphrodites that did not segregate Unc progeny were of genotype: *lon-1 sup-5*; *him-8*; *her-1(n827n695)*. A similar protocol was used to construct a *lon-1 sup-5*; *him-8*; *dpy-11 her-1(ct22n695)* strain. These strains were confirmed to contain *sup-5* by reisolating the *sup-5* mutation from each strain and demonstrating that it suppressed the *unc-13* amber allele *e1091*.

Strains containing *her-1(n827n695)* and *sup-7(st5)* or *her-1(ct22n695)* and *sup-7(st5)* were constructed as follows. *unc-13(e450) I*; *him-8(e1489) IV*; *sup-7(st5) X* males were crossed with *him-8(e1489)*; *dpy-11(e224) her-1(r) n695 V*; *lon-2(e678) X* hermaphrodites. From the progeny of the F_1 hermaphrodites, non-Unc non-Dpy non-Lon animals were picked. Animals that segregated Dpy's and ¼ Unc Lon's (that is, all Lon's were Unc) were of genotype *unc-13*; *him-8*; *dpy-11 her-1(r) n695/+ + +*; *lon-2/sup-7* [*sup-7* is a dominant suppressor of *unc-13(e450)*]. From the progeny of these hermaphrodites, Dpy non-Unc animals were picked. Those animals of genotype *unc-13*; *him-8*; *dpy-11 her-(r) n695*; *sup-7* were saved and scored for the appearance of intersex or male progeny.

Strains containing *sup-5(e1464)* and *her-1(y10)* [or *sup-5(e1464)* and *her-1(y14)*] were constructed as follows: *sup-5/unc-32*; *dpy-11 him-5/+ him-5* males were crossed with *unc-32*; *her-1 him-5* hermaphrodites. From the progeny of *sup-5/unc-32*; *her-1 him-5/dpy-11 him-5* hermaphrodites, L4 non-Unc, non-Dpy hermaphrodites were picked. Those hermaphrodites that segregated Unc's but not Dpy's were usually of genotype *sup-5/unc-32*; *her-1 him-5* and occasionally were of genotype *sup-5/unc-32*; *her-1 him-5/+ him-5*; the latter class produced Unc-32 male progeny. From the progeny of the former class, non-Unc hermaphrodites were picked. Those animals that did not segregate Unc's were of genotype *sup-5*; *her-1 him-5*. Such strains were confirmed to contain the *sup-5* mutation using methods described above.

A similar protocol was used to construct a *sup-5*; *him-8*; *her-1(e1807) sma-1* strain.

Generation of *nDf31*: The deficiency *nDf31* was isolated using the following protocol. L4 *n695* males were mutagenized with gamma irradiation (5000 rad) and mated with *unc-42 V*; *dpy-3 X* hermaphrodites. Unc-42 non-Dpy hermaphrodites (*i.e.*, cross progeny animals possibly containing a deficiency of the *unc-42* region) were picked from the cross progeny and scored for segregation of dead eggs or dead larvae (indicative of a recessive lethality, which is characteristic of deficiencies). One candidate segregated ¼ dead larvae; this lethality was linked to the *unc-42* region of LG V. The putative deficiency carried by this strain was designated *nDf31*.

Standard complementation tests with various markers on LG V were performed to determine the extent of the *nDf31* deficiency. For most complementation tests, *eT1/nDf31* males were crossed with hermaphrodites containing the marker in question and cross-progeny males were scored for the marker phenotype. Complementation tests with *her-1* were performed as described above. To test for complementation with *egl-3*, *nDf31/unc-23 sma-1* males were crossed with *egl-3 sma-1* hermaphrodites and the non-Sma cross progeny hermaphrodites were scored for the *egl-3* phenotype. To test for complementation with *daf-11(m47ts)*, *nDf31/eT1* males were crossed, at 16°, with *dpy-11 unc-42 daf-11* hermaphrodites and Unc non-Dpy (*nDf31/dpy-11 unc-42 daf-11*) cross progeny were picked and transferred to 20°. If *nDf31* fails to complement *daf-11*, then all viable progeny would be Daf. The partial genetic map of LG V shown in Figure 1 indicates the extent of *nDf31*.

Other mapping experiments: *egl-3* was positioned between *unc-41* and *daf-11* based on the following three-factor crosses: (1) Two of four Egl non-Unc-42 recombinants picked from the progeny of *unc-42 egl-3/unc-41* heterozygotes segregated Unc-41 hermaphrodites; (2) three of 18 Egl non-Unc-23 recombinants picked from the progeny of *unc-23 egl-3/her-1 unc-41* heterozygotes segregated *her-1 unc-41* animals; and (3) eight of nine Egl non-Sma recombinants picked from the progeny of *egl-3 sma-1/unc-42 daf-11* heterozygotes segregated Daf non-Unc-42 animals.

mDf1 was tested for complementation with *egl-3* by crossing *mDf1/sma-8* males with *egl-3 sma-1* hermaphrodites and scoring the non-Sma hermaphrodite cross progeny for the Egl phenotype. *sma-8(n716) V* shows a dominant Sma and recessive lethal phenotype and maps between *unc-42* and *sma-1* on LG V (PARK and HORVITZ 1986). *mDf3* was tested by crossing *mDf3/sma-1* males with *egl-3 sma-1* hermaphrodites and scoring the non-Sma hermaphrodites for the Egl phenotype. *sDf29* was tested for complementation with *unc-41* by crossing *sDf29/eT1* males with *dpy-11 unc-41* hermaphrodites and scoring non-Dpy cross progeny for the Unc phenotype. Complementation with *egl-3* was tested by crossing *sDf 29/eT1* males with *dpy-10*; *unc-23 egl-3* hermaphrodites and scoring Unc non-Dpy cross progeny hermaphrodites (*dpy-10/+;unc-23 egl-3/sDf29*) for the *egl-3* phenotype. For *mDf1*, *mDf3* and *sDf29*, complementation with *daf-11* was performed as described for *nDf31*.

Mating assays: Assays to determine the efficiency of male mating were performed as described by HODGKIN (1983). Six L4 males or six young adult males were placed with six L4 *dpy-11* hermaphrodites on a Petri dish seeded with a small circle of *Escherichia coli*. After about 24 hr the males were removed. Hermaphrodites were transferred to fresh plates daily, and total cross progeny were counted. Total cross progeny for one such experiment using adult males was 3148 for *n695 XO* males and 2885 for wild-type males.

RESULTS

***n695* intersexual and abnormal male animals:** The phenotype resulting from the mutation *n695* is variable: most animals are egg-laying defective (Egl) hermaphrodites, but about one in five is an obvious intersex or an abnormal male. The *n695* Egl hermaphrodites are self-fertile, but abnormal in the release of progeny. Some appear otherwise phenotypically normal, but many are clearly intersexual and exhibit an obviously masculinized tail. Animals with the most extreme *n695* phenotype have a male body shape and size and an abnormal male tail, but have never been observed to show male mating behavior; these animals rarely produce any self progeny. Representative *n695* phenotypes are illustrated in Figures 2 and 3.

The *n695* mutation is weakly temperature-sensitive: in homozygous animals, the mutation is greater than 90% penetrant at 20° and 25°, but only about 70% penetrant at 16°. The range of phenotypes observed is the same at all three temperatures. The *n695* mutation is incompletely dominant. Of the 50% of *n695/+* heterozygotes that show a mutant phenotype, most are Egl hermaphrodites; approximately one in twenty

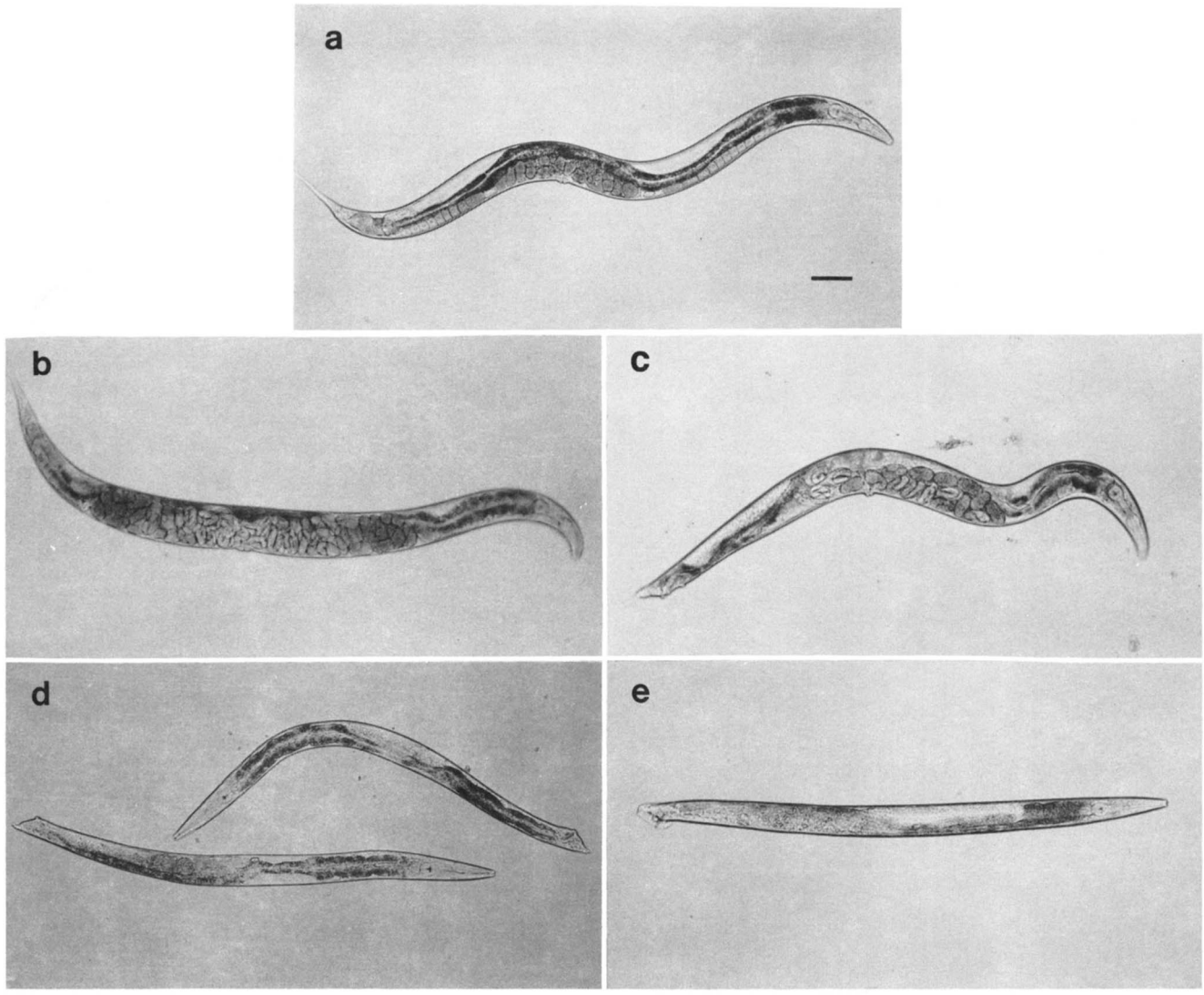

FIGURE 2.—Bright field photomicrographs of (a) a wild-type *XX* hermaphrodite, (b–d), *her-1(n695) XX* animals and (e) a *her-1(n695) XO* male. Many *n695 XX* animals are egg-laying defective hermaphrodites, which become very bloated with progeny as older adults but appear otherwise as normal hermaphrodites (compare a and b). Some *n695 XX* animals are more obviously intersexual, exhibiting both hermaphroditic (*e.g.*, presence of vulva or self-fertility) and male (*e.g.*, tail morphology) characteristics (c). Other *n695 XX* animals appear essentially as males with abnormal tail anatomy (d, top animal). *n695 XO* males are phenotypically wild-type males (e) (bar = 0.1 mm).

exhibits an intersexual or abnormal male phenotype.

***n695* is a transformer mutation:** We performed three experiments to determine whether *n695* intersexual and abnormal male animals are *XX* or *XO* in genotype.

1. Spontaneous males resulting from *X* chromosome nondisjunction normally arise among the self-progeny of wild-type hermaphrodites at a frequency of about 0.2% (HODGKIN, HORVITZ and BRENNER 1979). *him* (for high incidence of males) mutations have been identified that increase this frequency significantly; for example *him-5(e1467)* hermaphrodites produce about 16% self-progeny males (HODGKIN, HORVITZ and BRENNER 1979). If the *n695* intersex and abnormal male animals (which occur at a frequency of about 20%) are *XO* animals arising from increased *X* chromosome non-disjunction, then *n695* animals should be producing nullo-*X* gametes at a frequency similar to that of *him-5* and significantly higher than that of the wild type. The frequencies of nullo-*X* ovum production by *n695* and *him-5(e1467)* animals were determined as described in MATERIALS AND METHODS. These experiments indicate that *n695* hermaphrodites produce nullo-*X* ova at a frequency of <0.1% as compared to 12% for *him-5(e1467)* hermaphrodites. Therefore, nullo-*X* ovum production by *n695* hermaphrodites does not account for the intersex and abnormal male *n695* animals.

2. *n695 XO* animals were generated by constructing a double mutant strain containing *n695* and *him-5*. This strain produces phenotypically wild-type males (see Figures 2 and 3) as well as abnormal males. These wild-type males are *XO* in genotype: when crossed to *lon-2 X* hermaphrodites, they produce Lon (therefore

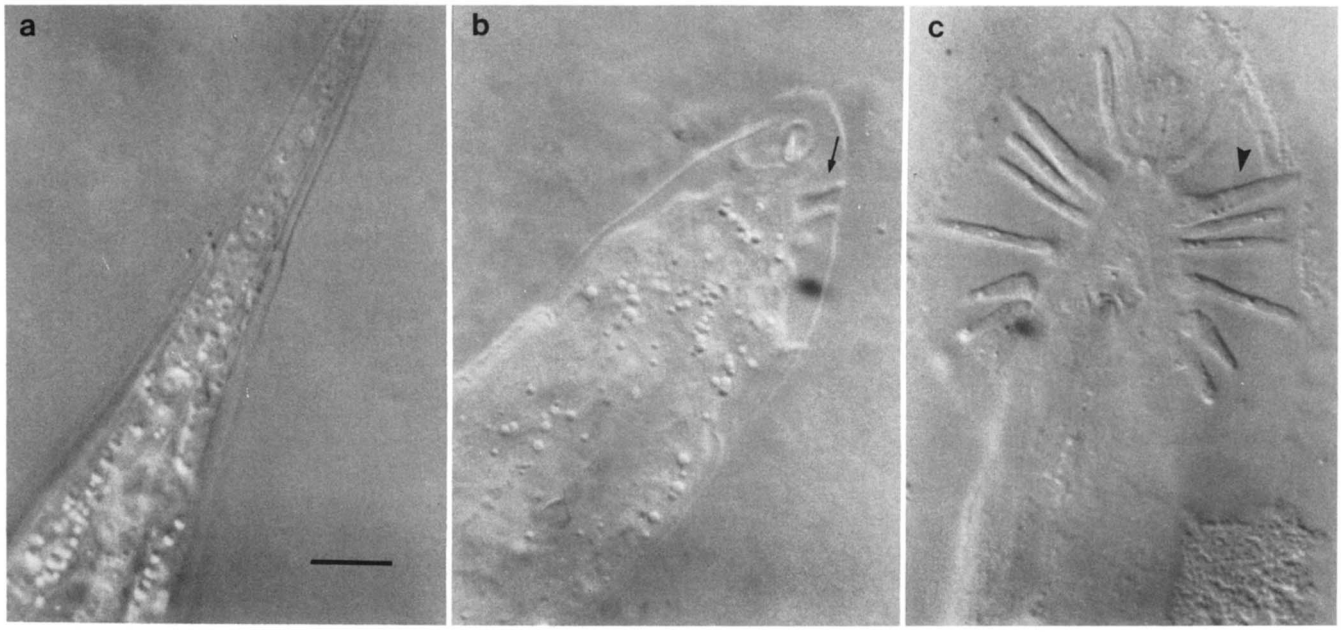

FIGURE 3.—Photomicrographs taken with Nomarski optics illustrating the tails of (a) a wild-type hermaphrodite (lateral view), (b) a masculinized *her-1(n695) XX* animal (dorsal view), and (c) a *her-1(n695) XO* male (dorsal view). *n695 XO* males are phenotypically wild-type males. [The male-specific sensory rays are indicated by an arrowhead. See SULSTON, ALBERTSON and THOMSON (1980) for a detailed description of male tail anatomy.] Note the presence of a few, shortened sensory rays in the *n695 XX* tail (arrow) (bar = 0.01 mm).

XO) male cross progeny as well as non-Lon *XX* hermaphrodite cross progeny. Furthermore, when *n695 XO* males are crossed with *dpy-10 II*; *n695 V*; *lon-2 X* hermaphrodites, all Lon (*n695 XO*) cross-progeny animals are wild-type males with respect to sexual phenotype, whereas non-Lon (*n695 XX*) cross progeny exhibit the intersexual or abnormal male phenotype (*n695*; *lon-2 XX* animals exhibit the *n695* phenotype). In mating assays, *n695 XO* males produced about the same number of cross progeny as wild-type males (see MATERIALS AND METHODS).

3. Mutations in the gene *dpy-21* cause *XX* animals to be dumpy in body shape (Dpy), and *XO* animals to be non-Dpy, regardless of sexual phenotype (HODGKIN and BRENNER 1977). Double mutant strains containing *n695* and a *dpy-21* mutation were constructed. For both the *e428* and *e459* alleles of *dpy-21*, the *n695 dpy-21* double mutant strain exhibits a less severe sexually transformed phenotype than *n695* by itself. Dpy (*XX*) animals are often Egl, but the obviously intersexual phenotypes are rarely observed. Nevertheless a few Dpy (therefore *XX*) abnormal males have been observed in these strains. Non-Dpy (*XO*) *n695 dpy-21* animals appear to be phenotypically wild-type males. (Mutations in other *dpy* genes tested, such as *dpy-11* and *dpy-3*, showed no such suppression of the *n695* phenotype.)

These observations demonstrate that the *n695* intersexual and abnormal male animals are *XX* in genotype. Thus, *n695* is a transformer mutation resulting in masculinization of *XX* animals and with no obvious effects on *XO* animals.

Identification of recessive alleles of the gene defined by *n695*: The dominant nature of *n695* suggests that it results in a gain-of-function at the locus defined by this mutation, because most dominant mutant phenotypes do not result from reduction or elimination of gene function (*e.g.*, PARK and HORVITZ 1986). In both *C. elegans* and Drosophila, the phenotypes resulting from dominant mutations that cause the overproduction or alteration of a gene product have been reverted by induction of null mutations at the locus (GREENWALD and HORVITZ 1980; GREENWALD, STERNBERG and HORVITZ 1983; HAZELRIGG and KAUFMAN 1983; PARK and HORVITZ 1986). Thus, the generation of phenotypic revertants of *n695* might identify null or reduction-of-function mutations at the *n695* locus. Such revertant strains were identified and characterized as described below.

Phenotypic revertants of *n695* were obtained by screening F_1 and F_2 progeny of mutagenized *n695* animals for non-Egl, non-Tra hermaphrodites. Twenty-two revertant strains were identified and are listed in MATERIALS AND METHODS. Most of these strains are essentially wild-type with respect to hermaphrodite sexual phenotype, although some exhibit a variable Egl phenotype.

Each of the revertant strains was examined for linkage of the suppressor mutation to *n695*. For all but five, the *n695* mutation could easily be recovered from the strain, indicating the existence of an unlinked or weakly linked extragenic suppressor mutation. Properties of these extragenic suppressors will

be reported elsewhere. The *n695* mutation could not be easily recovered from revertant strains containing the suppressor mutations *n826*, *n827*, *n830*, *n1100*, and *ct22*. For this reason, these strains were likely to carry intragenic suppressors of *n695* and were characterized in detail.

Linkage of each of the putative intragenic suppressor mutations to *n695* was determined as described in MATERIALS AND METHODS. All were very closely linked to *n695*: recombination between *n826* and *n695* and *n827* and *n695* was estimated to be <0.07%; between *ct22* and *n695* <0.04%; between *n830* and *n695* <0.06%; and between *n1100* and *n695* <0.1%. For comparison, the recombination distance across the gene *unc-54* (a myosin structural gene) or the gene *unc-22* is approximately 0.02% (WATERSTON, SMITH and MOERMAN 1982; MOERMAN and BAILLIE 1979).

The phenotypes resulting from these mutations are as follows. *XX* animals of genotype *n826n695*, *n827n695*, and *ct22n695* are hermaphrodites in sexual phenotype; *n830n695 XX* animals exhibit a variably small body size and *n1100n695 XX* animals are variably Egl, but otherwise both are hermaphrodite in sexual phenotype. The effects of these mutations in *XO* animals were initially examined by constructing strains containing *him-8* and the *n695* suppressor mutation (*e.g.*, *him-8*; *ct22n695*). [*him-8* hermaphrodites segregate about 37% *XO* self-progeny (HODGKIN, HORVITZ and BRENNER 1979).] In such strains carrying *n827n695* or *ct22n695*, no phenotypic males are observed. In such strains carrying *n826n695*, abnormal males and intersexual animals as well as a few wild-type males are observed at 25°, but only wild-type males at 16°. Variable small males with wild-type or slightly abnormal tails are observed at 25° for the *n830n695* strain, but only wild-type males are observed at 16°. (In mating assays, *n830n695* males grown at 25° mate very poorly.) Males that are wild type in appearance are observed in the *n1100n695* strain at 16° or 25°.

The above observations suggested that the *n827* and *ct22* suppressor mutations result in transformation of *XO* animals into phenotypic hermaphrodites. This hypothesis was confirmed by crossing *n827n695/+ +* and *ct22n695/+ +* males with *dpy-11 n827n695*; *lon-2 X* and *dpy-11 ct22n695*; *lon-2 X* hermaphrodites, respectively, and observing that Lon (*n827n695/n827n695 XO* and *ct22n695/ct22n695 XO*) hermaphrodites were produced. The *n826* and *n830* mutations appeared to result in weaker, temperature-sensitive transformations of *XO* animals into abnormal males or (in the case of *n826*) intersexual animals and hermaphrodites. To further characterize *n826n695* and *n830n695 XO* animals, phenotypically wild-type *n826n695* and *n830n695* males (grown at 16° or 20°) were crossed with *m;n826n695;lon-2 X* or *m; n830n695;lon-2 X* hermaphrodites, respectively. (*m* represents a recessive, visible mutation used to distinguish self-progeny from cross-progeny.) All Lon non-M progeny from such crosses are *XO* animals of genotype *n826n695/n826n695* or *n830n695/n830n695*. For *n826n695*, at 25°, the sexual phenotype of *XO* animals ranges from wild-type male to hermaphrodite; at 16° and 20° most *XO* animals are wild-type males. Such *n830n695 XO* animals, at 25°, are males, many of which appear slightly abnormal. At 16°, *n830n695 XO* animals are wild-type males. *n1100n695 XO* animals (identified as Lon Unc progeny of the cross: *him-8/+*; *n1100n695 unc-42/+ + +* males mated with *dpy-10;n1100n695 unc-42;lon-2* hermaphrodites) are phenotypically wild-type *XO* males at 16° and 25°. Table 1 summarizes these phenotypes.

***n695* is an allele of *her-1 V*:** We have mapped the *n695* mutation to the left of and close to *unc-42* on Linkage Group V (see MATERIALS AND METHODS for data). As seen by the partial genetic map of LG V shown in Figure 1, the map position of *n695* is very similar to that of the gene *her-1*. Recessive alleles of the *her-1* gene, originally described by HODGKIN (1980), transform *XO* animals into phenotypic hermaphrodites and have no apparent effect on *XX* animals.

We have tested each of the five putative intragenic suppressor mutations for complementation with either or both of the *her-1* alleles *e1518* or *e1520*, as described in MATERIALS AND METHODS. *n826n695*, *n827n695* and *ct22n695* fail to complement these *her-1* mutations, such that *sup n695/her-1 XO* animals are hermaphrodites. Thus these suppressor mutations are alleles of the *her-1* gene, consistent with the observations described above that demonstrate obvious Her phenotypes resulting from the *n826*, *n827* and *ct22* mutations. The *n830n695* mutation also fails to complement *e1520*, such that at 25° *n830n695/e1520 XO* animals show a weak Her phenotype: they are intersexual and usually exhibit a male tail, a hermaphrodite body size and an abnormal gonad with oocytes and sperm. Similarly, the *n1100n695* mutation fails to complement *e1520*, such that at 25° *n1100n695/e1520 XO* animals are usually males with variably abnormal gonads, some of which contain oocyte-like cells; occasionally, an *n1100n695/e1520 XO* animal exhibits a more extreme transformation. Thus both *n830* and *n1100* are alleles of *her-1*, although neither *n830n695* nor *n1100n695 XO* homozygotes are obviously transformed.

The very close linkage of *n695* and these *her-1* suppressor mutations suggests that the latter are intragenic suppressors of *n695* and, therefore, that *n695* is an allele of *her-1*. Furthermore, the recessive *her-1* [designated *her-1(r)*] mutations are *cis*-dominant suppressors of *n695*: the dominant *XX* transformer phe-

TABLE 1

Phenotypes of *XO* animals with various *her-1* genotypes

her-1 mutation (*m*)	*m/m*	*m/e1520*	*m/ct22n695*	*m/Df*
ct22n695	♀	♀	♀	♀
n827n695	♀	♀	♀	♀
n826n695 25°	WT ♂, ABN ♂, ISX, ♀	♀	♀	♀
n826n695 16°	WT ♂*	WT ♂, ABN ♂, ISX, ♀	♀	♀
n830n695 25°	ABN ♂	ABN ♂, ISX	ABN ♂, ISX	ISX, ♀
n830n695 16°	WT ♂*	WT ♂	WT ♂	WT ♂, ABN ♂
n1100n695 25°	WT ♂	WT ♂, ABN ♂, ISX	WT ♂**, ABN ♂, ISX	WT ♂, ABN ♂, ISX, ♀
n1100n695 16°	WT ♂	WT ♂	WT ♂	WT ♂

XO animals of the various *her-1* genotypes were generated as described in MATERIALS AND METHODS. Most of the scoring of sexual phenotype was performed using a Wild dissecting microscope. For some of the experiments, representative animals were scored using Nomarski optics. For *n826n695*, if >85% of the *XO* animals fell into one of the categories described below (*e.g.*, wild-type male), then only that category is listed.

The sexual phenotype of *XO* animals was assigned to one of the following four categories. (1) WT ♂: wild-type male. Such males appeared to be wild type in overall morphology, but subtle defects might not have been detected with the methods used. If marked with an asterisk (*), the males were known to be fertile and to produce cross progeny. (2) ABN ♂: abnormal male. These animals are very male-like in morphology but have obvious gonad or tail abnormalities. (3) ISX: intersexual animal. Such intersexes exhibit a combination of male and hermaphrodite features. For example, an animal that was self-fertile with a masculinized tail would fall into this category. In some cases, the distinction between an abnormal male and an intersexual animal was somewhat arbitrary. (4) ♀: hermaphrodite. Included in this category were animals that had both an hermaphrodite body size and shape and an hermaphrodite tail. Such animals were not necessarily observed to be self-fertile, but those with grossly abnormal gonads were included in the intersex category.

Df: chromosome carrying a deficiency of *her-1*. For *n826n695* and *n830n695*, all four deficiencies shown in Figure 1 were tested. For these alleles, the results were similar with all of the deficiencies. *nDf31*, *mDf1* and *mDf3* were tested with the *n1100n695* allele. The range of sexual phenotypes observed varied with the deficiency used. *nDf31/n1100n695* and *mDf1/n1100n695 XO* animals were generally more transformed than *mDf3/n1100n695 XO* animals.

** *n1100n695/ct22n695 XO* animals were generated by crossing *ct22n695/+ +* males with *dpy-10; n1100n695 unc-42; lon-2* hermaphrodites. Lon non-Unc cross-progeny were *XO* animals of genotype *n1100n695 unc-42/ct22n695* or *n1100n695 unc-42/+ +*. Sexually transformed *XO* animals were assumed to be of genotype *n1100n695/ct22n695*. From such an experiment, wild-type *XO* males could be of either genotype.

notype is suppressed when a *her-1(r)* mutation exists in *cis* (*e.g.*, all *ct22n695/+ +* or *n827n695/+ + XX* heterozygotes are wild-type hermaphrodites) but not in *trans* (*e.g.*, some *+ n695/e1917 +* or *+n695/e1520+ XX* heterozygotes are mutant) with *n695*. This evidence strongly indicates that the *her-1(r)* suppressors are intragenic. If the *her-1(r)* mutations were closely linked, extragenic dominant suppressors of *n695*, then one would expect *her-1(r) n695/+ +* heterozygotes to be phenotypically identical to *+ n695/her-1(r) +* heterozygotes. Thus *n695* appears to be a dominant allele of the *her-1* gene with phenotypic effects opposite to those of the previously characterized recessive *her-1* mutations.

***her-1(r)* mutations result in reduction of *her-1* function:** The following observations indicate that the phenotype of *her-1(r)* mutants results from reduction or loss of *her-1* gene function.

1. The *her-1(r)* mutations are recessive to the wild-type allele; such mutations usually result in a decrease of gene activity (MULLER 1932).

2. The *her-1(r)* mutations *n826* and *n827* were generated by the reversion of *n695* at a frequency of approximately 2×10^{-4} per gamete after standard EMS mutagenesis; this frequency is approximately that expected for the elimination of gene function (5×10^{-4} per mutagenized gamete) (BRENNER 1974; GREENWALD and HORVITZ 1980).

3. Each of the deficiencies *mDf1*, *mDf3* and *sDf29* appears to span the *her-1* locus, as each fails to complement mutations in the genes *unc-23* and *unc-42*, which flank *her-1* (Figure 1). We have examined the sexual phenotypes of various *her-1(r)/Df XO* heterozygotes. For *her-1(r)* alleles that result in complete transformation of all *XO* animals, such as *e1518*, *e1520*, *e1917*, *n827n695*, *ct22n695* and *y10*, the phenotypes of *her-1(r)/mDf1 XO* heterozygotes are the same as that of the *her-1(r) XO* homozygotes: *XO* animals are self-fertile hermaphrodites. (For some alleles, *XO* heterozygotes with *mDf3* or *sDf29* were also examined with the same results.) This observation suggests that *her-1(r)* mutations result in reduction and possibly complete loss of *her-1* gene function.

To examine further whether such alleles represent null gene function, we have tested the *n695* revertants *n827n695* and *ct22n695* for suppression by the amber suppressors *sup-5* (WILLS *et al.* 1983) and *sup-7* (WATERSTON 1981). When *him-8; her-1; sup* strains were constructed and scored for the presence of males or intersexual animals, no males or intersexes were observed in either case, indicating a lack of suppression. HODGKIN (1980; personal communication) has tested ten of the previously defined *her-1(r)* mutations (including *e1518*, *e1520*, *e1914* and *e1917*) for suppression by *sup-5* and two (*e1807* and *e1821*) for suppression by *sup-7* and has shown that none is

suppressed. In addition, we have shown that neither *her-1(e1807)*, *her-1(y10)*, nor *her-1(y14)* is suppressed by *sup-5*.

We have also examined the sexual phenotypes of *her-1(r)/Df XO* heterozygotes for the alleles *n826n695*, *n830n695* and *n1100n695*, which do not result in complete transformation of all *XO* animals (Table 1). *n826n695/Df XO* animals are hermaphrodites at 16° or 25°. For *n830n695/Df*, *XO* animals are sterile hermaphrodites, intersexes or (occasionally) abnormal males at 25° and wild-type or abnormal males at 16°. For *n1100n695/Df*, *XO* animals are variably transformed at 25° and their sexual phenotype ranges from wild-type male to sterile hermaphrodite; at 16° such *XO* animals appear to be wild-type males. Thus for all three mutations, *XO* deficiency heterozygotes are more severely transformed than *XO* homozygotes. This result is consistent with *n826n695*, *n830n695* and *n1100n695* being weak, partial loss-of-function alleles of *her-1*, exhibiting an obvious dosage dependence not observed with alleles such as *e1520* and *ct22n695*.

The weak *her-1(r)* alleles provided the opportunity to compare, under different conditions, the effect of a deficiency of *her-1* with the effects of different mutations in the gene. For example, *n826n695/Df XO* heterozygotes were compared with *n826n695/her-1(r) XO* heterozygotes at 16° (Table 2). The *n826n695/Df XO* heterozygotes are usually self-fertile hermaphrodites, although sterile hermaphrodites or intersexual animals are occasionally observed. However, for several of the *her-1(r)* mutations tested, the *n826n695/her-1(r) XO* heterozygotes are generally less transformed, ranging from essentially wild-type males to self-fertile hermaphrodites. In contrast, most *ct22n695*, *e1917* and *y10 XO* heterozygotes are self-fertile hermaphrodites, indicating that these *her-1* mutations behave most like a *her-1* deficiency in this assay.

A similar experiment was performed comparing *n830n695/Df XO* heterozygotes with *n830n695/her-1(r) XO* heterozygotes at 25°. The range of phenotypes of such *XO* animals is somewhat variable from experiment to experiment. In general, *n830n695/Df XO* animals range from abnormal males to sterile hermaphrodites with many in the latter category. For all *her-1(r)* mutations tested (including *ct22n695*, *e1917* and *y10*), *n830n695/her-1(r) XO* usually appear as grossly abnormal males or intersexual animals although hermaphrodites are sometimes observed (Table 1).

The *n695* mutation results in a gain of function at the *her-1* locus: The *her-1(r)* and *her-1(n695)* alleles result in opposite phenotypic effects: the recessive alleles transform *XO* animals into phenotypic hermaphrodites and have no obvious effects on *XX* animals, whereas the *n695* mutation transforms, with variable expressivity, *XX* animals into phenotypic males and has no effects on *XO* animals. Since the *her-1(r)* phenotype results from a reduction of gene function, it seems likely that the *her-1(n695)* phenotype results from a gain of function, that is, increased or novel gene activity. As discussed above, the dominant nature of the *n695* mutation also implies a gain-of-function at the *her-1* locus.

To test this hypothesis further, an experiment designed to delete the *her-1* region in a strain containing the *n695* mutation was performed. If *n695* is a gain-of-function mutation, heterozygotes of such deficiencies should not exhibit the transformed phenotype of *n695* heterozygotes, that is, the dominant *n695* phenotype should be reverted by a deletion of the locus itself. Such a deficiency, called *nDf31*, was generated in an *n695* background as described in MATERIALS AND METHODS. The extent of this deficiency is shown in Figure 1: *nDf31* fails to complement *her-1(r)*, *unc-42* and *unc-41* and complements *dpy-11*, *unc-23*, *egl-3*, *daf-11* and *sma-1*. *nDf31/+* heterozygotes do not

TABLE 2

Summary of *n826n695/m* (*her-1* mutation) and *n826n695/Df XO* phenotypes

m or *Df*	Temperature (°C)	Percent in category				*n*
		WT ♂	ABN ♂	ISX	♀	
n826n695	25	20	37	14	30	177
n826n695	20	87	11	1	<1	414
n826n695	16	99	<1	0	0	286
nDf31	16	<1	<1	5	94	354
mDf3	16	0	0	6	94	140
mDf1	16	<1	4	6	90	283
sDf29	16	0	0	3	97	71
ct22n695	16	<1	4	7	88	746
y10	16	1	6	8	85	603
e1917	16	1	5	13	81	361
e1914	16	2	7	14	77	481
e1559	16	3	13	13	71	390
e1519	16	9	12	10	70	555
e1807	16	2	11	20	66	295
e1518	16	10	13	19	58	633
e1564	16	15	27	14	45	183
y14	16	18	24	24	34	207
n827n695	16	31	25	19	25	223
e1520	16	37	36	11	16	1218
e1821	16	31	39	16	14	617
e1574	16	32	38	21	10	312
*e1561*ts	16	96	3	<1	0	126

Sexual phenotype was scored using a Wild dissecting microscope. The categories of sexual phenotypes are as described in the legend to Table 1. *n*: number of animals counted.

n826n695 XO animals were identified as the Lon non-Unc animals that result from crossing either *n826n695* or *him-8*; *n826n695* males with *unc-36*; *n826n695*; *lon-2* hermaphrodites. *n826n695/Df XO* animals were generated as described in MATERIALS AND METHODS. *XO* animals heterozygous for *n826n695* and another *her-1* allele were generated by crossing *n826n695* or *him-8*; *n826n695* males with *m*; *her-1*; *lon-2* hermaphrodites. *m* was either *dpy-10*, *dpy-11*, *unc-5*, *unc-36* or *sma-1*. All Lon non-M animals resulting from such a cross are *n826n695/her-1 XO* heterozygotes.

exhibit the transformed phenotype characteristic of many *n695/+* heterozygotes; thus, the generation of a deficiency of the *her-1* gene has reverted the dominant *n695* phenotype. In addition, *mDf1/+*, *mDf3/+* and *sDf29/+* heterozygotes do not exhibit the transformer phenotype of *n695/+* heterozygotes. (These deficiencies were generated in a *her-1(+)* background.) Since *mDf1*, *mDf3* and *sDf29* all span the *her-1* locus (see Figure 1), it is clear that complete loss of *her-1* function does not result in a dominant *n695* transformer phenotype.

We have examined the phenotypes of *n695/+*, *n695/her-1(r)* [where *her-(r)* is *e1520*, *e1518* or *e1917*] and *n695/Df* XX heterozygotes. For all three classes of heterozygotes, the penetrance of the *n695* Egl phenotype is much lower than that observed for *n695* homozygotes. The penetrance of the Egl phenotype in such heterozygotes is variable from experiment to experiment. For this reason, we have been unable to use the relative severity of the mutant phenotypes for the three classes of heterozygotes to analyze the nature of the *n695* mutation. We have observed that many *n695/Df XX* heterozygotes are wild-type hermaphrodites. This result indicates that *n695/Df XX* heterozygotes are less mutant than *n695 XX* homozygotes, consistent with the hypothesis that the *n695* mutation results in a gain-of-function in the *her-1* gene.

DISCUSSION

The dominant mutation *n695* results in an incomplete and variable transformation of *C. elegans XX* animals into phenotypic males and has no obvious effect on *XO* animals. In contrast, recessive alleles of the gene *her-1* [*her-1(r)* alleles] previously characterized by HODGKIN (1980), result in the opposite phenotype: *XO* animals are transformed into phenotypic hermaphrodites and *XX* animals are apparently unaffected. The experiments described in this paper and summarized below demonstrate that *n695* is a gain-of-function mutation in the *her-1* gene, causing a transformer phenotype opposite to that caused by the recessive *her-1(r)* alleles.

The similar map position of the *n695* mutation and the *her-1* gene initially suggested to us that *n695* might be allelic to recessive mutations in the *her-1* gene. To test this prediction, we isolated twenty-two revertant strains of *n695*; all exhibited wild-type hermaphrodite sexual phenotype in *XX* animals. Five of these revertant strains contained a suppressor mutation that was linked to *n695* and all five failed to complement the recessive *her-1* alleles *e1520* or *e1518*. These new *her-1(r)* mutations (*n826n695*, *n827n695*, *n830n695*, *n1100n695* and *ct22n695*) are intragenic suppressors of *n695* based on two observations: (1) the *her-1* mutations are closely linked to *n695* (*e.g.*, <0.04% for *ct22*), and (2) the *her-1* mutations are *cis*-dominant suppressors of *n695*, that is, the dominant transformer phenotype is suppressed when a recessive *her-1* mutation exists in *cis*, but not in *trans*, with *n695*.

The recessive *her-1* mutations generated by *n695* reversion vary widely with respect to the degree of transformation of *XO* animals. Both *ct22n695* and *n827n695* result in complete transformation of *XO* animals into phenotypic hermaphrodites. *n826n695* is a temperature-sensitive allele that results in variable transformation of *XO* animals at 25°. For both the *n830n695* and the *n1100n695* alleles, sexual transformation of *XO* animals is obvious only in *XO* heterozygotes of genotypes *n830n695/Df* and *n1100n695/Df* or *n830n695/her-1(r)* and *n1100n695/her-1(r)*.

The recessive nature of the *her-1(r)* mutations suggests that they result in a reduction of *her-1* function. Two additional lines of evidence support this hypothesis. First, the *her-1* mutations *n826* and *n827* were generated by EMS mutagenesis at a frequency of 2×10^{-4} per gamete, about that expected on the average for loss of gene function. Second, deficiency heterozygotes carrying *her-1(r)* alleles such as *ct22n695*, *e1917* and *e1520* [*e.g.*, *her-1 (ct22n695)/mDf1*] exhibit the same *XO* phenotype as the respective *her-1(r)* homozygotes, suggesting that such alleles are approximately equivalent to a deletion of the *her-1* locus and thus display the phenotype that results from severely reduced *her-1* gene function. Deficiency heterozygotes carrying the *her-1(r)* alleles *n826n695*, *n1100n695* and *ct22n695* [such as *her-1(n826n695)/mDf1*] exhibit more severe *XO* transformation than the respective *her-1(r) XO* homozygotes. The degree of *XO* sexual transformation is thus dose dependent for such alleles, suggesting that they represent partial loss of gene activity. One striking example is that at 16° two doses of the *n826n695* alelle in *XO* animals results in a wild-type male phenotype, whereas one dose (*n826n695/Df*) *in XO* animals results in hermaphrodite development. Thus, at 16° a twofold difference in dosage of this allele results in complete sexual transformation. This observation is interesting in light of a recently proposed dosage model for mammalian sex determination in which a single active dose of a sex-determining locus results in female development, while two active doses of this locus result in male development (PAGE *et al.* 1987).

The *her-1* alleles *ct22n695*, *y10* and *e1917* are the best candidates for null mutations in the *her-1* gene. In experiments comparing the phenotype of various *n826n695/her-1(r)* and *n826n695/Df XO* heterozygotes, the *ct22n695*, *e1917* and *y10* mutations behaved similarly to a deficiency of the *her-1* locus. In contrast, many of the other *her-1* mutations were not equivalent to a deficiency under these conditions. We suggest that the latter *her-1* alleles, which include the canoni-

cal *her-1* allele *e1520*, result in severe reduction but not complete loss of *her-1* gene function. In experiments comparing the phenotype of *n830n695/her-1(r)* and *n830n695/Df XO* heterozygotes, the latter are generally more transformed than the former, even when the *ct22n695*, *y10* or *e1917* allele is used as *her-1(r)*. Such an observation suggests that either (1) none of the existing *her-1* alleles (including *ct22n695*, *y10* or *e1917*) is null, or (2) these alleles are null but physical deletion of the *her-1* gene and adjacent regions of the chromosome has an effect on sexual phenotype that is not strictly equivalent to that seen with elimination of *her-1* gene function.

The opposite phenotypic effects of the dominant *n695* mutation and the recessive *her-1(r)* mutations suggest that if the *her-1(r)* phenotype results from loss of function, then the *n695* phenotype results from an increased function of the *her-1* locus. Increased *her-1* function might result from an overproduction of the gene product or from ectopic or constitutive gene activity. Three observations demonstrate that *n695* is such a gain-of-function mutation: (1) the deficiencies *mDf1*, *mDf3* and *sDf29*, which span the *her-1* locus, do not exhibit the dominant transformer phenotype of *n695*. This result indicates that the *n695* phenotype does not result from null gene function; (2) the dominant transformer phenotype of *n695* was reverted by a deletion (*nDf31*) of the *her-1* gene. The reversion of dominant mutations by gene deletion has been used in both *Drosophila* and *C. elegans* to identify gain-of-function mutations (LIFSCHYTZ and GREEN 1979; GREENWALD and HORVITZ 1980; HAZELRIGG and KAUFMAN 1983); and (3) *n695/Df XX* heterozygotes are clearly less transformed than *n695 XX* homozygotes, in contrast to observations with the recessive *her-1* alleles. Such an observation is inconsistent with the *n695* mutation causing a loss of function.

Based on epistatic interactions between mutations in the various *tra*, *fem* and *her* genes, HODGKIN (1986) has proposed a model for the control of sex-determination in *C. elegans*. Briefly, sexual phenotype is determined by the state of the *tra-1* gene, which is controlled primarily by the *her-1*, *tra-2*, *tra-3* and *fem* genes. When the *tra-1* gene is ON (that is, when active gene product is present), male development is repressed and hermaphrodite development is activated. When this gene is OFF, that is, active gene product is absent, male development occurs. HODGKIN (1980) has proposed that the activity of the *her-1* gene is controlled by the ratio of *X* chromosomes to sets of autosomes (*X:A* ratio). If the *X:A* ratio is high (as in *XX* animals), the *her-1* gene is OFF, which causes (through a series of negative regulatory interactions involving *tra-2*, *tra-3* and the *fem* genes) the *tra-1* gene to be ON, so that hermaphrodite development ensues. If the *X:A* ratio is low (as in *XO* animals), *her-1* gene activity is ON, *tra-1* is OFF, and male development occurs.

Our data are consistent with this model of sex determination. If *her-1* is required for male development and is normally OFF in *XX* animals and ON in *XO* animals, then a loss-of-function mutation in this gene would cause both *XX* and *XO* animals to develop as wild-type *XX* animals, that is, as hermaphrodites. Such a phenotype is exhibited by the candidate null *her-1(r)* mutations such as *ct22n695*. HODGKIN's model would predict that expression of active *her-1* gene product is sufficient to stimulate male development, such that increased, ectopic or constitutive *her-1* activity in *XX* animals would lead to expression of male characteristics. *n695* animals exhibit such a phenotype. Thus, the *n695* mutation appears to result in increased, ectopic or constitutive *her-1* activity in *XX* animals.

The partial transformation observed in *n695 XX* animals probably results from levels of *her-1* gene activity that are lower than in wild-type *XO* animals but higher than in wild-type *XX* animals. An alternative possibility is that the partial male phenotype of *n695* results from an aberrant activity (or abnormal functioning) of the *her-1* gene product, rather than from intermediate levels of "normal" (*i.e.*, with respect to function in *XO* animals) *her-1* activity. However, the observation that *n695 XO* animals are wild-type males argues against this interpretation, because the *n695* gene product apparently can function normally in *XO* animals to induce wild-type male development. Thus, at least with respect to the stimulation of male development, *n695* has "normal" rather than aberrant function, consistent with the hypothesis that the *n695* mutation results in a failure in *XX* animals to regulate properly the level of *her-1* activity. The *n695* allele appears to still be regulated at least to some extent by the *X:A* ratio, since the *n695 XX* transformer phenotype is suppressed in *2A;3X* hermaphrodites (TRENT 1982).

The genetic and phenotypic characteristics of the recessive and dominant alleles of *her-1* parallel those observed for alleles of the gene *tra-1* (HODGKIN and BRENNER 1977; HODGKIN 1980, 1987). Recessive, loss-of-function alleles of *tra-1* transform *XX* animals into phenotypic males, whereas dominant, gain-of-function alleles exhibit an opposite transformation of *XO* animals. The genes *her-1* and *tra-1* appear to be control genes involved in specifying sexual identity and subsequent sex-specific development: both genes are defined by two classes of mutations that lead to opposite changes in gene function (loss-of-function *vs.* gain-of-function) resulting in opposite phenotypic transformations. The existence of such opposite classes of mutations for a particular gene is important for distinguishing a control gene from genes with

products that may be necessary for differentiation but are not involved in specifying developmental decisions *per se* [see STERNBERG and HORVITZ (1984) and GREENWALD (1985) for further discussion]. Similar observations have been made identifying opposite classes of alleles of the genes *tra-2* (HODGKIN and BRENNER 1977; DONIACH 1986), *fem-3* (HODGKIN 1986; BARTON, SCHEDL and KIMBLE 1987), *lin-12* (GREENWALD, STERNBERG and HORVITZ 1983) and *lin-14* (AMBROS and HORVITZ 1984, 1987) of *C. elegans*. Recessive and dominant alleles of each of these genes have opposite effects on the specification of cell fates in a number of tissues. This type of control gene, for which the level of activity can determine developmental fate, may function in many developmental decisions in *C. elegans*.

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LITERATURE CITED

AMBROS, V., and R. HORVITZ, 1984 Heterochronic mutants of the nematode *Caenorhabditis elegans*. Science **226:** 409–416.

AMBROS, V., and R. HORVITZ, 1987 The *lin-14* locus of *Caenorhabditis elegans* controls the time of expression of specific postembryonic developmental events. Genes Dev. **1:** 398–414.

BAKER, B., and J. BELOTE, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*. Annu. Rev. Genet. **17:** 345–393.

BARTON, M. K., T. SCHEDL and J. KIMBLE, 1987 Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. Genetics **115:** 107–119.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

DONIACH, T., 1986 Activity of the sex-determining gene *tra-2* is modulated to allow spermatogenesis in the *C. elegans* hermaphrodite. Genetics **114:** 53–76.

DONIACH, T., and J. HODGKIN, 1984 A sex-determining gene, *fem-1*, required for both male and hermaphrodite development in *Caenorhabditis elegans*. Dev. Biol. **106:** 223–235.

EDGLEY, M., and D. RIDDLE, 1987 *Genetic Maps*, Vol. 4, Edited by S. J. O'BRIEN. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

EICHER, E., and L. WASHBURN, 1986 Genetic control of primary sex determination in mice. Annu. Rev. Genet. **20:** 327–360.

GREENWALD, I., 1985 The genetic analysis of cell lineage in *Caenorhabditis elegans*. Phil. Trans. R. Soc. Lond. B **312:** 129–137.

GREENWALD, I., and R. HORVITZ, 1980 *unc-93(e1500)*: a behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

GREENWALD, I., P. STERNBERG and R. HORVITZ, 1983 The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. Cell **34:** 435–444.

HAZELRIGG, T., and T. KAUFMAN, 1983 Revertants of dominant mutations associated with the Antennapedia gene complex of *Drosophila melanogaster*: cytology and genetics. Genetics **105:** 581–600.

HERMAN, R., and R. HORVITZ, 1980 Genetic analysis of *Caenorhabditis elegans*. pp. 227–261. In: *Nematodes as Biological Models*, Edited by B. ZUCKERMAN. Academic Press, New York.

HODGKIN, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HODGKIN, J., 1983 Male phenotypes and mating efficiency in *Caenorhabditis elegans*. Genetics **103:** 43–64.

HODGKIN, J., 1986 Sex determination in the nematode *C. elegans*: analysis of *tra-3* suppressors and characterization of *fem* genes. Genetics **114:** 15–52.

HODGKIN, J., 1987 A genetic analysis of the sex-determining gene, *tra-1*, in the nematode *Caenorhabditis elegans*. Genes Dev. **1:** 731–745.

HODGKIN, J., and S. BRENNER, 1977 Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. Genetics **86:** 275–287.

HODGKIN, J., R. HORVITZ and S. BRENNER, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

HORVITZ, R., S. BRENNER, J. HODGKIN and R. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

KIMBLE, J., and D. HIRSH, 1979 The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Genetics **70:** 396–417.

KIMBLE, J., L. EDGAR and D. HIRSH, 1984 Specification of male development in *Caenorhabditis elegans*: the *fem* genes. Dev. Biol. **105:** 234–239.

LIFSCHYTZ, E., and M. GREEN, 1979 Genetic identification of dominant overproducing mutants: the beadex gene. Mol. Gen. Genet. **171:** 153–159.

MENEELY, P., and W. WOOD, 1984 An autosomal gene that affects *XO* chromosome expression and sex determination in *Caenorhabditis elegans*. Genetics **106:** 29–44.

MOERMAN, D., and D. BAILLIE, 1979 Genetic organization in *Caenorhabditis elegans*: fine structure analysis of the *unc-22* gene. Genetics **91:** 95–103.

MULLER, H., 1932 Further studies on the nature and causes of gene mutations. pp. 213–255. In: *Proceedings of the Sixth International Congress of Genetics*.

PAGE, D., R. MOSHER, E. SIMPSON, E. FISHER, G. MARDON, J. POLLACK, B. MCGILLIVRAY, A. DE LA CHAPELLE and L. BROWN, 1987 The sex-determining region of the human *Y* chromosome encodes a finger protein. Cell **51:** 1091–1104.

PARK, E. C., and R. HORVITZ, 1986 Mutations with dominant effects on the behavior and morphology of the nematode *Caenorhabditis elegans*. Genetics **113:** 853–867.

RIDDLE, D., M. SWANSON and P. ALBERT, 1981 Interacting genes in dauer larva formation. Nature **290:** 668–671.

ROSENBLUTH, R., and D. BAILLIE, 1981 The genetic analysis of a reciprocal translocation, *eT1(III;V)*, in *Caenorhabditis elegans*. Genetics **99:** 415–428.

ROSENBLUTH, R., C. CUDDEFORD and D. BAILLIE, 1985 Mutagenesis in *Caenorhabditis elegans*. II. A spectrum of mutational events induced with 1500 R of gamma-radiation. Genetics **109:** 493–511.

STERNBERG, P., and R. HORVITZ, 1984 The genetic control of cell lineage during nematode development. Annu. Rev. Genet. **18:** 489–524.

SULSTON, J., and R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

SULSTON, J., D. ALBERTSON and J. THOMSON, 1980 The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. **78:** 542–576.

SULSTON, J., E. SCHIERENBERG, J. WHITE and N. THOMSON, 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans.* Dev. Biol. **100:** 64–119.

TRENT, C., 1982 Genetic and behavioral studies of the egg-laying system of *Caenorhabditis elegans.* Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, Mass.

TRENT, C., N. TSUNG and R. HORVITZ, 1983 Egg-laying defective mutants of the nematode *Caenorhabditis elegans.* Genetics **104:** 619–647.

WATERSTON, R., 1981 A second informational suppressor, *sup-7 X,* in *Caenorhabditis elegans.* Genetics **97:** 307–325.

WATERSTON, R., and S. BRENNER, 1978 A suppressor mutation in the nematode acting on specific alleles of many genes. Nature **274:** 715–719.

WATERSTON, R., K. SMITH and D. MOERMAN, 1982 Genetic fine structure analysis of the myosin heavy chain gene *unc-54* of *Caenorhabditis elegans.* J. Mol. Biol. **158:** 1–15.

WILLS, N., R. GESTELAND, J. KARN, L. BARNETT, S. BOLTEN and R. WATERSTON, 1983 The genes *sup-7 X* and *sup-5 III* of *Caenorhabditis elegans* suppress amber nonsense mutations via altered transfer RNA. Cell **33:** 576–583.

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